

To identify activators, which generally act as positive gating modulators, a lower Ca^{2+} concentration (~200 nM) was used. The QPatch results were benchmarked against manual patch-clamp electrophysiology by determining the potency of several commonly used KCa3.1 inhibitors (TRAM-34, NS6180, ChTX) and activators (EBIO, riluzole, SKA-31). Collectively, our results demonstrate that the QPatch provides a comparable but much faster approach to study compound interactions with KCa3.1 channels in a robust and reliable assay.

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Diversity in the Pharmacological Profile of Heterotetrameric Kv2/KvS Channels for Channel Blockers

Jeroen I. Stas, Elke Bocksteins, Alain J. Labro, Dirk J. Snyders.
University of Antwerp, Antwerp, Belgium.

Voltage-gated K^+ (Kv) are tetramers of α -subunits each consisting of 6 trans-membrane segments (S1-S6) and a cytoplasmic N- and C-terminus. The S5-S6 segments of each subunit assemble to generate the central pore while the S1-S4 segments form the voltage-sensing domains. The PXP motif in the middle of S6 provides a degree of flexibility to the bottom half of the S6 segment which is necessary for channel gating. This region is also critical for the interaction with channel blockers. Based on sequence homology, eight *Shaker*-related Kv subfamilies have been identified: Kv1-Kv6 , Kv8-Kv9 . The silent (KvS) subunits (Kv5-Kv9) cannot form homotetramers but assemble with Kv2 subunits into Kv2/KvS heterotetramers that display unique biophysical properties. KvS subunits lack the 2nd proline residue of the PXP motif which may impact on the pharmacological profile of channel blockers. We tested this hypothesis by using the Kv1.5(P511G) mutant in which the 2nd proline of the PXP motif was replaced by a glycine. Homotetrameric Kv1.5(P511G) channels were insensitive to 4-AP while heterotetrameric $\text{Kv1.5-Kv1.5(P511G)}$ channels (stoichiometry controlled by using dimers), still displayed current inhibition. However, $\text{Kv1.5-Kv1.5(P511G)}$ channels were significantly less sensitive displaying an IC_{50} values of 16 mM instead of 270 μM for wild type (WT) Kv1.5 . Similarly, heterotetrameric Kv2/KvS channels displayed an altered affinity for 4-AP compared to WT Kv2.1 ; 18 mM (IC_{50} for Kv2.1) inhibited 17%, 60%, 82% and 13% of Kv5.1 , Kv6.3 , Kv8.1 and Kv9.3 -containing currents, respectively. Furthermore, the heterotetrameric Kv2/KvS channels displayed also a subtle change in the affinity for the open channel blockers quinidine and flecainide. These results suggest that the absence of a complete PXP motif in one or two out of four subunits alters the pharmacological profile. (Supported by FWO fellowships to JS and EB & grant FWO-G.0449.11N to DJS).

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Subunit Composition Determines $\text{G}\beta\gamma$ Activation of Single Girk Channels

Daniel Yakubovich^{1,2}, Nathan Dascal¹.

¹Tel-Aviv University, Sackler School of medicine, Tel-Aviv, Ramat Aviv, Israel, ²The Edmond and Lily Safra Children's Hospital, Ramat-Gan, Israel. GIRK (G-protein activated inward rectifier potassium channels) are direct effectors of $\text{G}\beta\gamma$ expressed in cardiac myocytes, neurons and other cells. GIRKs modulate resting membrane potential and mediate inhibitory actions of many neurotransmitters. GIRKs are involved in heart rate inhibition by vagus nerve, long term potentiation, pain mediation, drug addiction, etc. These channels are tetramers and GIRK1/2 is most abundant in brain while GIRK1/4 is mainly expressed in cardiomyocytes. We compared the kinetic single-channel properties of GIRK1/2 and GIRK1/4. Channels were expressed in *Xenopus* oocytes with $\text{G}\beta\gamma$ subunit of G-proteins. The dose of $\text{G}\beta\gamma$ was chosen to produce maximal activation, as verified in whole-cell recordings. GIRK channels in cells co-expressing $\text{G}\beta\gamma$ demonstrated robust activity in cell-attached membrane patches. Recordings demonstrating no overlaps for long episodes of activity (~ 5-10 min) were considered as single channel and were subjected to detailed kinetic analysis. We found that maximal open probability ($P_{o,max}$) of GIRK1/4 ($0.05 \pm .01$, $n = 5$) is almost 3 fold lower than that of GIRK1/2 (0.15 ± 0.027 , $n = 6$). Mean closed time of GIRK1/4 (35.88 ± 6.6 ms, $n = 5$) was significantly longer than of GIRK1/2 (14.74 ± 3.58 ms, $n = 6$). No significant difference was found between the mean open times. Closed times distribution of GIRK1/4 was satisfactorily fitted with 5 exponents (as found previously in excised patches), whereas only 4 exponents were sufficient for fitting the closed times distribution of GIRK1/2. It was hypothesized that GIRK channel as a tetramer can be found in 5 closed states (0-4 bound $\text{G}\beta\gamma$). The fact that only 4 closed states are populated in GIRK1/2 can point to higher affinity to $\text{G}\beta\gamma$ compared to GIRK1/4. Analysis of published literature supports lower EC_{50} values for GIRK1/2 than for GIRK1/4 observed in dose-response to $\text{G}\beta\gamma$ in excised patches.

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G-Protein Activated Inwardly Rectifying Potassium Channels Control Motility of Breast Cancer Cells

Simin Rezaei¹, Chouyang Li¹, Sarah Kammerer¹, Astrid Gorischek¹, Trevor Devaney¹, Amir Hassan Zarnani², Thomas Bauernhofer³, Wolfgang Schreibmayer¹.

¹Department of Biophysics, Medical University of Graz, Graz, Austria,

²Immunology Research Center, Iran University of Medical Sciences, Tehran, Iran, Islamic Republic of, ³Oncology Department, Medical University of Graz, Graz, Austria.

Mortality of breast cancer is very high when it metastasizes to distant organs. New early tumor markers and mechanistic insight into carcinogenesis and metastasis are needed to treat the disease at curable stages. GIRK channels are activated by GPCRs and regulate resting potential and excitability of neurons, myocytes and other cells. Recently GIRK proteins have been associated with endocrine adenomas and also breast cancer (Stringer et al. 2001, Brevet et al. 2008 and Choi et al. 2009). In this study we analyzed the vital parameters of cancerous and noncancerous breast cell lines when human GIRK1 and splice variants (hG1a, hG1c, hG1d) are overexpressed or silenced.

GIRK1a, GIRK1c and GIRK1d were stably overexpressed and knocked out in breast cell lines MCF7, MDA-MB-231 and MCF 10A. Vital parameters including invasion, wound healing, adhesion, proliferation and motility were evaluated.

Our findings revealed that overexpression of GIRK1a significantly increased the velocity 0.102 ± 0.035 ($\mu\text{m}/\text{min}$) in overexpressed vs. 0.05 ± 0.02 in vector control cells ($P < 0.001$) and motility coefficient 0.2 ± 0.3 ($\mu\text{m}^2/\text{min}$) in overexpressed vs. 0.05 ± 0.06 in vector control cells ($P < 0.001$) of the MCF7 cell lines. Overexpression of hG1c in MCF7 significantly increased invasiveness of the cells ($P < 0.001$) as measured by matrigel assay. Overexpression of the GIRK1 and splice variants didn't have significant effect on proliferation, wound healing and adhesion of the MCF7 cells.

Stably overexpression of GIRK1 and splice variants in MCF7 cause increased velocity, motility coefficient and invasion of the cells. Expression of GIRK might be a new diagnostic biomarker of breast cancer and possibly is causally involved in metastasis.

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Maximal Activity of KcsA, KirBac1.1 and Kir2.1 Channels are Differentially Regulated by Membrane Thickness

Benoit Mondou, Louis Sasseville, Jean-Louis Schwartz, Jurgen Sygusch, Nazzareno D'Avanzo.

Biochemistry, Université de Montréal, Montreal, QC, Canada.

Ion channels may be regulated by numerous factors, including the physicochemical properties of the membrane in which they are imbedded. Hydrophobic matching between the hydrophobic thickness of the bilayer and the channel's hydrophobic length is thought to minimize the energetic penalty that would be needed to solvate hydrophobic residues or exposed lipid tails. Here we examine the role of hydrophobic matching in regulating the activity of 3 potassium channels, KcsA, KirBac1.1 and human Kir2.1. Purified channels were reconstituted into membranes containing 25 mol% POPG and 75 mol% $\text{diC}_{18:1}\text{PC}$ ($14 < n < 22$) (+ 1 mol% $\text{PI}(4,5)\text{P}_2$ for Kir2.1). $^{86}\text{Rb}^+$ influx assays indicate that KcsA channels are maximally active in thick membranes ($\text{diC}_{20:1}\text{PC}$), while both KirBac1.1 and Kir2.1 channels were maximally active in thinner membranes (eg. $< \text{diC}_{18:1}\text{PC}$). Single channel recordings in lipid bilayers of the same compositions indicate that membrane thickness affects the open probability of KcsA, but not unitary conductance. SAXS was used to quantify the hydrophobic thickness of each of our experimental conditions in order to quantify the energy associated with hydrophobic matching for these 3 proteins. Our initial calculations suggest the energies associated with membrane stretching or curvature are too great to account for hydrophobic matching in these channels, and suggest tilting of the α -helices of the proteins are required for these channels to be maximally active. Molecular dynamic simulations provide further insight into the molecular details associated with hydrophobic matching for each of these channels.

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Activation of Slack Channels Alters their Interactions with the Pp1 Targeting Protein Pactrl

Matthew R. Fleming¹, Leonard K. Kaczmarek².

¹Pharmacology, Yale University, New Haven, CT, USA, ²Pharmacology and Cellular and Molecular Physiology, Yale University, New Haven, CT, USA.

Slack Na^+ -activated K^+ channels contribute to neuronal adaptation during sustained stimulation and regulate the temporal accuracy of action potentials.